


FORM PTO-1390 (REV 10-95)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
<b>TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371</b>				F7414(C)	
				U.S. APPLICATION NO. (If known, see 37 CFR §1.5)	
INTERNATIONAL APPLICATION NO. PCT/EP98/08552		INTERNATIONAL FILING DATE 23 DECEMBER 1998		PRIORITY DATE CLAIMED 22 January 1998	
TITLE OF INVENTION FROZEN FOOD PRODUCT					
APPLICANT(S) FOR DO/EO/US CHENEY, Paul, et al.					
<p><b>Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:</b></p> <ol style="list-style-type: none"> <li>1. <input checked="" type="checkbox"/> This is a <b>FIRST</b> submission of items concerning a filing under 35 U.S.C. §371.</li> <li>2. <input type="checkbox"/> This is a <b>SECOND</b> or <b>SUBSEQUENT</b> submission of items concerning a filing under 35 U.S.C. §371.</li> <li>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).</li> <li>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19<sup>th</sup> month from the earliest claimed priority date.</li> <li>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. §371(c)(2))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US).</li> </ol> </li> <li>6. <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. §371(c)(2)).</li> <li>7. <input type="checkbox"/> A copy of the International Search Report (PCT/ISA/210).</li> <li>8. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))             <ol style="list-style-type: none"> <li>a. <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</li> <li>b. <input type="checkbox"/> have been transmitted by the International Bureau.</li> <li>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</li> <li>d. <input checked="" type="checkbox"/> have not been made and will not be made.</li> </ol> </li> <li>9. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).</li> <li>10. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).</li> <li>11. <input type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/ISA/409).</li> <li>12. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).</li> </ol> <p><b>Items 13. to 19. below concern document(s) or information included:</b></p> <ol style="list-style-type: none"> <li>13. <input type="checkbox"/> An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.</li> <li>14. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.</li> <li>15. <input checked="" type="checkbox"/> A FIRST preliminary amendment.             <ol style="list-style-type: none"> <li><input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</li> </ol> </li> <li>16. <input type="checkbox"/> A substitute specification.</li> <li>17. <input type="checkbox"/> A change of power of attorney and/or address letter.</li> <li>18. <input type="checkbox"/> Certificate of Mailing by Express Mail</li> <li>19. <input type="checkbox"/> Other items or information:</li> </ol>					



RECEIVED JUL 21 2000

U.S. APPLICATION NO. (If known, see 37 CFR §1.4)		INTERNATIONAL APPLICATION NO.		ATTORNEY'S DOCKET NUMBER	
09/600788		PCT/EP98/08552		F7414(C)	
17. <input checked="" type="checkbox"/> The following fees are submitted: <b>BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)):</b> Search Report has been prepared by the EPO or JPO..... \$840.00 International preliminary examination fee paid to USPTO (37 CFR §1.482)..... \$670.00 No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2))..... \$760.00 Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO..... \$970.00 International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... \$96.00  <div style="text-align: right;"><b>ENTER APPROPRIATE BASIC FEE AMOUNT =</b> \$840.00</div>				<b>CALCULATIONS</b> PTO USE ONLY	
Surcharge of <b>\$130.00</b> for furnishing the oath or declaration later than months from the earliest claimed priority date (37 C.F.R. §1.492(e)). <input type="checkbox"/> 20 <input type="checkbox"/> 30				\$0.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	9 - 20 =	0	x \$ 18.00	\$0.00	
Independent claims	1 - 3 =	0	x \$ 78.00	\$0.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$ 260.00		
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$840.00	
Reduction of 1/2 for filing by small entity, if applicable. A Verified Small Entity Statement must also be filed (Note 37 C.F.R. §§1.9, 1.27, 1.28).					
<b>SUBTOTAL =</b>				\$840.00	
Processing fee of <b>\$130.00</b> for furnishing the English translation later than months from the earliest claimed priority date (37 C.F.R. §1.492(f)). <input type="checkbox"/> 20 <input type="checkbox"/> 30					
<b>TOTAL NATIONAL FEE =</b>				\$840.00	
Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). \$40.00 per property.					
<b>TOTAL FEES ENCLOSED =</b>				\$840.00	
				Amount to be refunded:	
				charged:	
a. <input type="checkbox"/> A check in the amount of <u>\$840.00</u> to cover the above fees is enclosed. b. <input checked="" type="checkbox"/> Please charge my Deposit Account No. <u>12-1155</u> in the amount of <u>\$840.00</u> to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>12-1155</u> . A duplicate copy of this sheet is enclosed.					
<b>NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.</b>					
SEND ALL CORRESPONDENCE TO:					
Linda Horvath UNILEVER US INC. 45 River Road Edgewater, New Jersey 07020 US (201) 840-2333					
 SIGNATURE					
I, William Millen, Representative NAME					
119,544 REGISTRATION NUMBER					
Filed: July 21, 2000 AJZ:IWM:aek:k\job\lever\646x\nat\ phase transmtl					

International Application No. : PCT/EP98/08552  
International Filing Date : 23 December 1998  
Priority Date(s) Claimed : 22 January 1998  
Applicant(s) (DO/EO/US) : CHENEY, Paul, et al.  
Title: FROZEN FOOD PRODUCT :

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Although the above-identified International application has been amended under Article 34, applicants request that examination in the United States National Phase be based on the claims as originally filed in the International application, and this Preliminary Amendment is based thereon. Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend this application as follows:

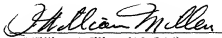
**IN THE CLAIMS:**

Claims 3 and 4, line 1:	Delete "or 2".
Claims 5 and 6, line 1:	Change "any preceding claim" to -- claim 1 --.
Claim 10, line 1:	Change "any one of claims 7 to 9" to -- claim 7 --.
Claim 11, line 1:	Change "any one of claims 7 to 10" to -- claim 7 --.

**Remarks**

The purpose of this Preliminary Amendment is to eliminate the multiple dependency of the claims in order to avoid the additional fee and to correct improper multiple dependencies. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Respectfully submitted,



I. William Millen (19,544)

Representative capacity

MILLEN, WHITE, ZELANO & BRANIGAN, P.C.

Arlington Courthouse Plaza I

2200 Clarendon Boulevard, Suite 1400

Arlington VA 22201

Direct Dial: 703-812-5325

Facsimile: 703-243-6410

E-mail: millen@mwzb.com

IWM: aek

FILED: July 21, 2000

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WO 99/37164

Frozen Food product

Technical Field of the Invention

- 5 The invention relates to food products containing Anti-freeze peptides (AFPs), in particular to frozen food products containing AFPs.

Background to the Invention

10

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs. In particular it has been suggested that some AFPs may be capable of increasing the smooth texture of frozen food products such as ice cream. Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the desired texture and eating characteristics.

20

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food products such as ice cream. No guidelines are given as to how to obtain smooth textures.

25

WO 92/22581 discloses AFPs from plants, which can be used for controlling ice crystal growth. This document also describes a process for extracting a polypeptide composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

30

- 2 -

Applicants believe that one of the possible reasons for the lack of desired texture in frozen food products containing AFP is that although the AFP is capable of recrystallisation inhibition it is often not capable of avoiding less favourable hard and brittle textures. Applicants believe that one of the explanations for this is that AFPs seem capable of controlling the growth of the ice-crystals. However the presence of AFP may also lead to an adverse effect in that the ice crystals tend to form aggregates leading to hard and brittle products. Thus the texture of the product is unfavourably affected during the manufacturing process.

The present invention therefore aims at defining manufacturing conditions which improve the texture of AFP containing frozen food products.

Surprisingly it has now been found that if the conditions for producing the frozen food material are carefully chosen, this leads to an improved texture. In particular applicants have found that if the production process includes the use of one or more of the following steps;

(i) a (pre-)freezing step which is a nucleation dominated freezing process;

(ii) a (post-)compaction step using a screw extruder or a (piston) compactor;

this leads to aggregation of the ice crystals being minimised and therefore results in more favourable textures of the frozen product, said texture being maintained for prolonged storage periods.

- 3 -

- Generally in the freezing of a composition, two distinct phases can be seen: at the onset of the freezing process many small ice crystals are rapidly formed. This phase is called the nucleation phase of the freezing process. Following the nucleation process the remaining part of the composition freezes onto the surface of the nucleates and therewith contributes to the growth of the ice crystals. This phase in the freezing process is called the growth phase. In a growth dominated freezing process most of the composition is frozen during the growth phase, in a nucleation dominated freezing process most of the composition is frozen during the nucleation phase.
- 15 Traditional freezing processes for frozen confectionery products for example involves the use of scraped surface heat exchangers whereby the mix to be frozen is subjected to shear during the freezing process. Generally this freezing process takes from 5 to 30 minutes for the product to reach
- 20 a temperature of  $-5^{\circ}\text{C}$  or lower. In this process initially some nucleation of ice crystals occurs followed by a period wherein ice crystal growth dominates.
- Alternative freezing processes, which for example are used
- 25 for the freezing of water ice involves the low shear or quiescent freezing of the mix, for example by filling a mould and dipping the mould into a cold bath, for example filled with brine. In this process initial nucleation of ice crystals takes place at the surface of the mould, the
- 30 internal of the product then tend to freeze more slowly in a freezing process dominated by growth.

- 4 -

Applicants have now surprisingly found that the aggregation in AFP containing products can significantly be reduced if a nucleation dominated freezing process is chosen. Such a freezing process is generally characterised by a short  
5 freezing time in combination with the formation of small ice crystals. Preferably the freezing process is carried out under low shear or quiescent freezing conditions.

10 Disclosure of the Invention

Accordingly, in a first aspect the present invention relates to a process for the manufacture of a frozen food product comprising AFPs, wherein the process comprises one or more  
15 of the following steps;

- (i) a (pre-)freezing step which is a nucleation dominated freezing process;
- 20 (ii) a (post-)compaction step using a screw extruder or a (piston) compactor;

For the purpose of this invention the term AFP has the meaning well-known in the art, see for example "Antifreeze  
25 proteins and their potential use in frozen food products", Marilyn Griffith and K. Vanya Ewart, Biotechnology Advances, Vol 13, pp 375-402, 1995.

The present invention aims at providing the food  
30 manufacturer with a greater flexibility for using AFP material in frozen food products when aiming to obtain a product with improved recrystallisation properties in combination with a good texture. In particular it has been



- 5 -

found that the texture of frozen food products containing AFPs can markedly be improved by carefully controlling its production method.

- 5 The invention is based on the finding that if the frozen product is produced by a process involving one or more of the following process steps;

(i) a (pre-)freezing step which is a nucleation dominated  
10 freezing process;

(ii) a (post-)compaction step using a screw extruder or a (piston) compactor;

- 15 this can lead to an improved texture of the product.

Applicants believe that it is well within the ability of the skilled person to select those freezing methods which result in a nucleation dominated freezing processes.

- 20 Preferably when the freezing process is a nucleation dominated freezing process, the freezing process is such that the product reaches a temperature of  $-5^{\circ}\text{C}$  or lower within 30 seconds, preferably in 0.01 to 25 seconds, most  
25 preferably in 1 to 15 seconds.

- Also preferably when the freezing process is a nucleated dominated freezing process, the freezing process is such that it results in many relatively small crystals, whereby  
30 the average size of the crystals is from 0.01 to 20 micrometer, more preferred from 0.01 to 15 micrometer most preferred from 0.01 to 10 micrometer.

- 6 -

For example rapid freezing processes tend to be nucleation dominated. Suitable processes may for example involve:

- (a) surface freezing, preferably film freezing onto a cold surface;
- (b) freezing of supercooled systems;
- (c) decompression freezing;
- (d) freezing by very low temperatures;
- (e) rapid particulate freezing, preferably condensation freezing.

Other rapid freezing processes will be apparent to the skilled person and are also embraced in the scope of the present invention. Preferably the freezing processes involve no or low shear.

Surface freezing preferably involves the application of a thin film or discrete particles onto a cold surface, optionally followed by removal of the frozen material. Preferably the film or particle thickness is from 0.01 to 5 mm. The cold surface is preferably at a temperature below -15°C, more preferred less than -20°C, most preferred less than -25°C. Suitably the surface can be cooled by applying liquid nitrogen, glycols or methanol. The removal can be done by any suitable means, for example by scraping, therewith producing frozen particles, for example flakes or pellets which can then be further processed. Obviously during further processing care should be taken to avoid substantial melting of the composition, which may result in growth dominated re-freezing.

In a very preferred embodiment surface freezing involves film freezing on a drum freezer which is for example cooled

- 7 -

with liquid nitrogen or methanol, followed by removal of the film from the drum freezer.

In a further embodiment of surface freezing a cryogenic plate freezer cooled with liquid nitrogen is used to produce frozen pellets.

An alternative form of surface freezing involves the preparation of a cold core followed by applying the mix to be frozen to the core for example by dipping or spraying, whereby a relatively thin film adheres to the cold core. Advantageously, such a cold core may for example be a very cold (e.g. dipped into liquid nitrogen) ice-cream core where upon a thin film of water-ice comprising AFP is frozen.

Another method of achieving rapid, nucleation dominated freezing is to produce a supercooled system at low temperatures followed by sudden freezing e.g. by applying a shock to the supercooled liquid. The rapid freezing of a supercooled liquid generally is a nucleation dominated freezing process.

Preferably the supercooled liquid has a temperature of at least 1 degree below the melting point, more preferred from 1-20 degrees below the melting point, for example 2-10 degrees below the melting point.

A third method of achieving rapid nucleation dominated freezing is to use decompression freezing. This involves the applying of high pressures to a liquid system while cooling it followed by removing the overpressure. This removal of the pressure then results in a rapid nucleation dominated freezing.

- 8 -

Preferably the pressure to be applied is from 100 to 3000 bar, for example from 200 to 2000, generally from 300 to 1300 bar. The temperature of the product before removing the  
5 over-pressure is preferably at least 5 degrees below the melting point at atmospheric pressure, preferably 6-10 degrees below the melting point.

A fourth method of ensuring nucleation dominated freezing is  
10 the application of very low temperatures. For example small drops of material to be frozen may be immersed into a fluid freezing medium e.g. liquid hexane or liquid nitrogen. Preferably the freezing temperature for this method is less than -50 °C. This method works best for relatively small or  
15 thin products to be frozen. Small products are preferably less than 5 grams, more preferred from 0.001 to 3 grams, most preferred 0.01 to 1 gram and may for example be drops of liquid immersed in the freezing medium. Relatively thin products may for example be sheets or thin streams of  
20 products, preferably having at least 1 dimension of less than 2 cm, more preferred 0.1 to 0.5 cm.

The product for use in this method may for example be directly immersed into the freezing liquid, alternatively  
25 however the products are first contacted with a protective layer e.g. filled into a mould, pumped through a pipe whereby these are contacted with the cooling medium.

A fifth preferred method to freeze food products of the  
30 invention involves rapid particulate freezing, preferably condensation freezing. This may for example be achieved by spraying a liquid mix to be frozen into a very cold gaseous environment or into a cooled environment. An especially

- 9 -

preferred method for rapidly freezing a liquid into particulates is condensation freezing. Most preferred is the use of techniques which are for example use in the production of artificial snow.

5

The production of artificial snow is widely described in the literature. Often artificial snow is produced in so called snow cannons whereby the water is frozen by spraying a mixture of water and pressurised air. Preferably the snowmaking takes place in an environment having a temperature of less than  $-3^{\circ}\text{C}$ , most preferred  $-5$  to  $-50^{\circ}\text{C}$  and a relative humidity of less than 75%, most preferred less than 50%.

15 Frozen particulates obtained by this fifth method can vary in size, but generally the number average diameter of the particles will be up to 10 mm, more preferred less than 5 mm. Generally each particulates will comprise multiple aggregated ice-crystals.

20

The freezing of frozen confectionery products by means of a screw extruder is for example described in: EP 713,650 (Societe des Produits Nestle), EP 410,512 (HMF Krampe & Co et al); EP 561,118 (Milchhof-Eiskrem GmbH et al),  
25 EP 351,476 (Goavec S.A. Societe Dite).

Preferably the manufacturing process of the invention involves the use of a screw extruder whereby the extrusion temperature of the frozen product is  $-8^{\circ}\text{C}$  or lower, more  
30 preferred from  $-10$  to  $-25^{\circ}\text{C}$ , most preferred from  $-12$  to  $-20^{\circ}\text{C}$ .

- 10 -

Suitable screw extruders for use in the process of the invention can for example be twin screw extruders such as described for example in EP 561,118. Also single screw extruders may be used. Also extruders may be used which  
5 combine more than one function of the ice-cream manufacturing process (see for example EP 713,650).

The conditions under which the screw extruder operates may vary for example depending on the type and size of the  
10 extruder used. Applicants believe that it is well within the ability of the skilled person to select those operating conditions such that a favourable quality of the product is obtained. Examples of suitable operating conditions are given in the examples.

15 Alternatively a compactor may advantageously be used in the manufacturing of frozen food products with AFPs. All suitable compactors such as presses may be used, especially preferred is the use of a piston compactors whereby pressure  
20 is applied onto the food products by means of the movement of a piston. Traditionally piston compactors have for example been used in the filling of sausages. Again applicants believe that it is within the ability of the skilled person to select the appropriate operating  
25 conditions of the (piston) compactor.

Preferably the invention relates to a process for the manufacture of a frozen food product comprising AFPs, wherein the process comprises the following steps;

30 (i) a (pre-)freezing step which is a nucleation dominated freezing process; and

- 11 -

(ii) a (post-)compaction step using a screw extruder or a (piston) compactor.

The use of a screw extruder or compactor may very advantageously applied to products which have been pre-frozen under such conditions that a particulate (partial) frozen material is produced for example flakes, pellets, powders, extended rods or sheets. For these pre-frozen products the use of screw extruders or (piston) compactors may advantageously lead to the compaction of the particulate material into a more solid structure.

The complete manufacturing process of the frozen products of the invention may comprise further optional steps in addition to pre-freezing and/or screw extrusion or piston compaction. For example the mixing of the ingredients, ageing, pasteurisation, homogenisation, pre-aeration etc. These optional steps can take place in any suitable order.

As described above, one of the characteristics of nucleation dominated freezing process is the formation of many small ice crystals. Applicants have found that the combined use of AFPs as an ingredient and nucleation dominated freezing process leads to a particular advantageous texture of the products to be frozen, said textures being maintained for long storage periods.

Particularly the nucleation dominated freezing process can very advantageously be used for the production of a particulate frozen material. Examples of these are frozen flakes, frozen (small) droplets, frozen powders, pellets, frozen rods and frozen snow. Surprisingly particulate materials formed by the process of the invention have a

- 12 -

reduced tendency to aggregation and therefore the free flowing nature of the particulate material can be maintained over storage, even if the storage temperature is relatively high.

5 Further the applicants have found that the use of a screw extruder or (piston) compactor in the production of AFP containing frozen food products is very advantageous in that it too can lead to very small ice-crystal sizes which can be  
10 maintained for long periods of storage.

Preferably the freezing conditions are chosen such that the average size of the ice crystals in the final frozen product is from 0.01 to 20 micrometer, said ice crystal sizes to be  
15 maintained in said range upon storage at -10 °C for 3 weeks.

Preferably the average ice crystal size remains less than 15 micrometer, for example less than 12 or even 10 micrometer during storage for 3 weeks at -10°C.

20 If the freezing process involves a nucleation dominated freezing process in the absence of any compaction process, the frozen product provided may be a particulate food product. If, on the other hand, a screw extruder or a  
25 (piston) compactor is used, products may be formed which are homogeneously solid and having no (fine) particulates.

Preferably non-particulate products of the invention have a smallest dimension of more than 2 cm, more preferred more  
30 than 2.5 cm, most preferred more than 3 cm.

After freezing the product may be further handled. For example the product may be filled into packages say of 0.05



- 13 -

to 10 litres and then stored. Alternatively the product may be further shaped or formed into the final product. For example the product can be used to the shaped into ice-cream gateaux.

5

A further advantage of the invention is that when the process used includes post compaction using either a screw extruder or a (piston) compactor, products of the invention generally do not need to be subjected to a hardening step, for example in a hardening tunnel. This advantage has for example been suggested for AFP products in general in US 5,620,732 (Pillsbury).

10

The process as described in US 5,620,732 however has as a disadvantage that this is not suitable for the production of luxury stick products. These products are traditionally made by extruding and cutting a block of ice-cream, hardening the block, followed by stick insertion and coating for example with chocolate or fruit water ice. If the hardening step is omitted for AFP containing products this leads to problems in the further handling for example during stick insertion or further coating.

15

20

Surprisingly applicants have found that the combined use of AFPs and post-compaction with either a screw extruder or (piston) compactor now renders it possible to product luxury stick products without the use of a hardening step.

25

Frozen food products of the invention may be any food product which can be stored and/or eaten in the frozen state. Examples of frozen food products which may contain AFPs are processed food products such as for example frozen bakery products for example dough, batters, cakes etc.,

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- 14 -

frozen culinary products for example soups, sauces, pizzas, frozen vegetable products such a compote, mashed potato, tomato paste etc.

- 5 Applicants have found that the method of the invention is best applicable to food systems which are fluid or liquid prior to freezing. A very preferred food product according to the invention is a frozen confectionery product.
- 10 For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, ice milk and frozen custard; as well as frozen confections which do not typically contain milk such as water ices, sorbet,
- 15 granitas and frozen fruit purees. Especially preferred products of the invention are ice-cream and water ice.

- Frozen products according to the invention may be aerated. For example the level of aeration is more than 50%, more
- 20 preferably more than 70%, most preferable more than 90%. Generally the level of aeration will be less than 400%, more general less than 300%, most preferred less than 200%. Aeration may for example take place prior or during freezing. If the product is pre-frozen by one or more of the
- 25 above described nucleation dominated freezing processes then preferably the aeration takes place prior to pre-freezing.

- Preferably the level of AFPs in the frozen food product of the invention is from 0.0001 to 0.5 wt% based on the final
- 30 product.

The AFP for use in products of the invention can be any AFP suitable for use in food products. Examples of suitable

- 15 -

sources of AFP are for example given in the above mentioned article of Griffith and Vanya Ewart and in patent applications WO 98/04699, WO 98/04146, WO 98/04147, WO 98/04148 and WO 98/22591.

5

The AFPs can be obtained from their sources by any suitable process, for example the isolation processes as described in the above mentioned documents.

- 10 One possible source of AFP materials is fish. Examples of fish AFP materials are AFGP (for example obtainable from Atlantic cod, Greenland cod and Tomcod), Type I AFP (for example obtainable from Winter flounder, Yellowtail flounder, Shorthorn sculpin and Grubby sculpin), Type II AFP
- 15 (for example obtainable from Sea raven, Smelt and Atlantic herring) and Type III AFP (for example obtainable from Ocean Pout, Atlantic wolffish, Radiated shanny, Rock gunnel and Laval's eelpout). A preferred example of the latter type is described in WO 97/02343.

20

Another possible source of AFP material are invertebrates. Also AFPs may be obtained from Bacteria.

- A third possible source of AFP material are plants. Examples
- 25 of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, daylily, winter barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern
- 30 cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

- 16 -

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs. Genetic manipulation techniques may be used to produce AFPs having at least 80%, more preferred more than 95%, most preferred 100% homology to the AFPs directly obtained from the natural sources. For the purpose of the invention these AFPs possessing this high level of homology are also embraced within the term "AFPs".

The genetic manipulation techniques may be used as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (for example in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell culture systems and plants all transformed with the appropriate expression vectors.

- 17 -

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our case WO 97/02343. From plants especially the use of AFPs from carrot or grass are preferred.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. This can be measured in accordance to Example I.

Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation, as measured in accordance to the examples, of less than 20  $\mu\text{m}$ , more preferred from 5 to 15  $\mu\text{m}$ .

Preferably the level of solids in the frozen food product (for example sugar, fat, flavouring etc.) is more than 2 wt%, more preferred from 4 to 70wt%.

The method of preparing the frozen food product of the invention can be selected from any suitable method for the preparation of frozen food products. The AFPs can generally be added at various stages of the preparation, for example it can be added in the first pre-mix of ingredients or can

- 18 -

later be added during a later stage of the preparation process. For some applications it is sometimes preferred to add the AFPs at a relatively late stage of the production process, for example after (partial) pre-freezing of the product.

The freezing process of the invention will generally include the freezing of the composition say to a temperature of less than  $-2^{\circ}\text{C}$ , say from  $-80$  to  $-5^{\circ}\text{C}$ . If desired, products of the invention do not need to be subjected to low temperatures to avoid ice-crystal growth. Therefore the products may for example be frozen without the need to use low temperatures say less than  $-25^{\circ}\text{C}$  and can also be stored at temperatures which are higher than traditional temperatures to store frozen confectionery products.

Preferably the freezing process involves conditions of low or no shear e.g. found in the freezing of filled moulds, dipping, thin film crystallisation, dropping into liquid nitrogen etc.

For some applications it may be advantageous to include a mixture of two or more different AFPs into the food product. One reason for this can for example be that the plant source for the AFPs to be used, contains more than one AFP and it is more convenient to add these, for example because they are both present in the AFP source to be used. Alternatively it may sometimes be desirable to add more than one AFP from different sources.

- 19 -

The invention will now be illustrated by means of the following examples.

**Example I**

5

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

Recrystallisation inhibition properties can be measured using a modified "splat assay" (Knight et al, 1988). 2.5  $\mu$ l of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16 mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at  $-80^{\circ}\text{C}$  in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the  $-80^{\circ}\text{C}$  hexane bath to the viewing chamber containing hexane held at  $-6^{\circ}\text{C}$  using forceps pre-cooled in the dry ice. Upon transfer to  $-6^{\circ}\text{C}$ , sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 60 minutes. The size of the ice-crystals in both assays is compared by placing the slides within a temperature controlled cryostat cabinet (Bright Instrument Co Ltd, Huntington, UK). Images of the samples are transferred to a Quantimet 520 MC image analysis system (Leica, Cambridge UK) by means of a Sony monochrome CCD videocamera. Ice crystal sizing was performed by hand-drawing around ice-crystal. At least 400 crystals were sized for each sample. The ice crystal size was taken as being the longest dimension of the 2D projection of each

- 20 -

crystal. The average crystal size was determined as the number average of the individual crystal sizes. If the size at 30-60 minutes is similar or only moderately (less than 10%) increased compared to the size at  $t=0$ , and/or the  
5 crystal size is less than 20 micrometer, preferably from 5 to 15 micrometer this is an indication of good ice-crystal recrystallisation properties



- 21 -

**Example II**

The following formulation:

- 15%wt sugar
- 5 10%wt skimmed milk powder
- 10%wt butterfat
- 0.2%wt locust bean gum
- 0.2%wt monglyceride
- 0.01%wt AFP\*
- 10 balance water

\* AFP HPLC 12 as described in WO 97/02343

- was produced using conventional ice-cream processing
- 15 equipment. The premix was cooled to 0°C
  - before passing through a Megatron model MT1-63/3A mixer,
  - operating at 8000 rpm. The mixer had a gap of 0.5mm between
  - the rotor and stator. An equal volume of air was injected
  - into the premix immediately before the mixing device. This
  - 20 gave an overrun of 90% in the premix.

- This aerated premix was frozen by applying a 0.5mm thickness
- on a Gerstenberg and Agger pilot cooling drum, having a
- surface area of 0.2m<sup>2</sup>, and operating at a rotational speed of
- 25 5 rpm. The drum was cooled with liquid Nitrogen. Frozen
  - flakes were removed using a plastic scraping knife after 1
  - revolution (i.e. after 12 seconds). The flakes had a
  - temperature of -20 °C. The flakes were collected, hardened in
  - a blast freezer at -35 °C, then stored at -25 °C.

30

The ice-cream flakes were soft and creamy.

- 22 -

The particle size of the ice crystals were determined as in Example I. The ice crystal size was well below 20 micron and remained below 20 micron after storage for 3 weeks at  $-10^{\circ}\text{C}$ .

- 23 -

**Example III**

An ice-cream premix of the formulation of Example II was produced using conventional ice-cream processing equipment.

- 5 The premix was cooled to 0 °C before passing through a Megatron model MT1-63/3A mixer, operating at 8000 rpm and with a gap of 0.5mm between the rotor and stator. An equal volume of air was injected into the premix immediately before the mixing device. This gave an overrun of 90% in the
- 10 premix.

- The aerated premix was pumped through a plate heat exchanger, whose coolant temperature was controlled to -7 °C, a temperature warmer than the metastable limit of -8 °C for
- 15 the premix. The premix exited the heat exchanger at -6 °C; the melting temperature of the premix was -2 °C. No ice was present in the premix, i.e. it was undercooled.

- The premix was poured into conventional metal ice-lolly
- 20 moulds, which were cooled by brine at -35 °C. Sticks were inserted into the moulds. After 15 minutes, the frozen ice-cream products were removed from the moulds.

- The products were stored at -25 °C. The ice-cream products
- 25 had a soft and creamy texture.

- 24 -

**Comparative Example IV**

An ice-cream premix of formulation of Example II was produced using conventional ice-cream processing equipment.

- 5 The premix was cooled to 0 °C before passing through a Megatron model MT1-63/3A mixer, operating at 8000 rpm and with a gap of 0.5mm between the rotor and stator. An equal volume of air was injected into the premix immediately before the mixing device. This gave an overrun of 90% in the
- 10 premix.

- The aerated premix was frozen in a standard ice-cream scraped surface heat exchanger (Crepaco W104, supplied by APV, operating with a series 80 dasher at a rotational speed
- 15 of 240 rpm) at a rate of 200 l/hr. The exit temperature was -5 °C, after a residence time of 90 seconds. The ice-cream was then hardened in a blast freezer at -35 °C, before storage at -25 °C.

- 20 The ice-cream was found to be hard and brittle.

**Example V**

A liquid premix for the preparation of ice-cream was  
5 prepared by mixing:

	<b>Ingredient</b>	<b>% by weight</b>
	Skimmed milk powder	10.00
	sucrose	13.00
10	maltodextrin (MD40)	4.00
	Locust bean gum	0.14
	butter oil	8.00
	monoglyceride (palmitate)	0.30
	vanillin	0.01
15	AFP**	0.01
	water	balance

\*\* AFP is carrot AFP prepared as follows (WO 98/2259).  
Carrots (*Daucus carota* cv Autumn King) were grown in  
20 individual pots. When plants were approximately twelve weeks  
old, they were transferred to a cold room and held at 4°C in  
constant light during 4 weeks for cold-acclimation. Plants  
were watered three times a week. Root extract from cold  
acclimatised carrot roots was prepared by scrubbing freshly  
25 pulled cold acclimatised (as described above) carrots in cold  
water. The tops were removed and the juice extracted  
employing a domestic juice extractor (Russell Hobbs, model no  
9915). The juice was frozen in 1 litre blocks and stored at  
-20°C prior to collection for use in ice cream formulations.

30

The composition was prefrozen to -5 °C and aerated to 100%  
overrun in a traditional scraped surface heat exchanger.

- 26 -

The composition was further frozen in a single screw extruder having a barrel length of 0.75 m, a diameter of 0.2 m a screw pitch of 0.135 m (2 start) and a screw channel depth of 15 mm.

5

The throughput was 280 kg/hour, the inlet pressure 7 barg and a constant torque of the screw was 1500Nm. The output pressure was 8 barg. The screw extruder was cooled such that the extrusion temperature was -12 °C

10

As a comparison (B) the same product was produced using a conventional scraped surface heat exchanger.

15 A comparison (C) the same product was produced by the bove screw extruder process, whereby the AFP was omitted from the formulation.

The resulting products were stored for 3 weeks at -10 °C.

20

Composition A had a smoother and creamier texture than formulations B and C.

- 27 -

**Example VI**

A liquid premix for the preparation of ice-cream was prepared by mixing:

5

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
maltodextrin (MD40)	4.00
10 Locust bean gum	0.14
butter oil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (of example V)	0.01
15 water	balance

The liquid mix was continuously aerated at a throughput of 60 litres/hour using a high speed rotor/stator mixer (megatron, Kinematica AG) to an overrun of 100%. the mix temperature was 5 °C and a mixer speed of 1600 rpm was employed. A pressure of 3 barg was maintained within the mixing head.

20

The aerated mix was then continuously spread as a 0.1 mm film on the surface of a drum freezer cooled with a methanol solution at -28 °C. The drum freezer was rotated at a rate of 1rpm. After one complete revolution the frozen film at -10 °C was removed continuously by means of a scraper blade to form frozen flakes.

30

The frozen flakes were compressed batch-wise using a piston compression device. The compressed ice cream was extruded

- 28 -

through a nozzle and packaged for storage. The ice crystal size distribution of the frozen material was measured as follows: by placing the coverplates smeared with the compositions to be tested within a temperature controlled cryostat cabinet (Bright Instrument Co Ltd, Huntington, UK). Images of the samples are transferred to a Quantimet 520 MC image analysis system (Leica, Cambridge UK) by means of a Sony monochrome CCD videocamera. Ice crystal sizing was performed by hand-drawing around ice-crystal. At least 400 crystals were sized for each sample. The ice crystal size was taken as being the longest dimension of the 2D projection of each crystal. The average crystal size was determined as the number average of the individual crystal sizes.

The average ice crystal size was 5.8 micrometer for the fresh sample with AFP and 7.2 micrometer for the fresh sample with AFP. After storage for 3 weeks at -10 °C the particle size of the sample with AFP was 7.7. micrometer, without AFP 43.2 micrometer.



**Example VII**

Example VI was repeated, but now the pre-frozen flakes are  
5 fed via an hopper to a twin screw extruder (CP1050, APV)  
which was cooled with a methanol solution at -28 °C. Co-  
rotating fully intermeshing screw rotors were fitted and a  
rotational speed of 10 rpm was used. The ice-cream was  
compressed and extruded at a temperature of -12 °C.

- 30 -

**Example VIII**

A liquid premix for the preparation of ice-cream was prepared by mixing:

5

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
maltodextrin (MD40)	4.00
10 Locust bean gum	0.14
butter oil	12.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP*	0.01
15 water	balance

\* AFP is AFP of HPLC-12 as described in WO 97/02343.

The mix was aerated to 100% overrun as in example VI. The  
20 aerated mix was frozen in the form of 10 mm diameter pellets  
using a cryogenic freezing unit (British Oxygen Company).  
The freezing surface consisted of a rotating horizontal  
turntable, which was cooled using liquid nitrogen to a  
temperature of -100 °C. The air above the freezing turntable  
25 was also cooled to a temperature of -120 °C. The turntable  
was rotated at 5rpm. After a single rotation the frozen  
pellets were swept off the freezing surface and collected.

The frozen pellets were then fed into a screw extruder under  
30 the same conditions as in Example VII.

- 31 -

**Claims**

1. A frozen food product comprising AFPs, said product having an average ice crystal size of 0.01 to 20 micrometer, wherein said crystal size is maintained between 0.01 and 20 micrometer upon storage for 3 weeks at -10°C.
2. A frozen food product according to claim 1 wherein the average ice crystal size after 3 weeks at -10°C is from 0.01 to 10 micrometer.
3. A frozen food product according to claim 1 or 2 being a particulate food product.
4. A frozen food product according to claim 1 or 2 being a non-particulate food product.
5. A frozen food product according to any preceding claim, wherein the frozen food product is a frozen confectionery product.
6. A product according to any preceding claim, wherein the product comprises 0.0001 to 0.5 wt% of AFP.
7. A process for the manufacture of a frozen food product comprising AFPs, wherein the process comprises one or more of the following steps;
  - (i) a (pre-)freezing step which is a nucleation dominated freezing step;
  - (ii) a (post-)compaction step using a screw extruder or a compactor.

- 32 -

8. A process according to claim 7 wherein in step (i) the product is frozen to a temperature of  $-5^{\circ}\text{C}$  or lower within 30 seconds.

5

9. A process according to claim 7 wherein in step (i) the product is frozen to a temperature of  $-5^{\circ}\text{C}$  or lower in 1 to 15 seconds.

10 10. A process according to any one of claims 7 to 9, wherein step (i) involves one or more of:

- (a) surface freezing, preferably film freezing, onto a cold surface;
- (b) freezing of a supercooled system;
- 15 (c) decompression freezing;
- (d) freezing at very low temperatures;
- (e) rapid particulate freezing, preferably condensation freezing.

20 11. A process according to any one of claims 7 to 10, wherein step (i) involves drum freezing of the product.

12. A process according to claim 7, wherein in step (ii) a screw extruder is used.

25 13. A process according to claim 12, wherein in step (ii) the extrusion temperature of the frozen food product is less than  $-8^{\circ}\text{C}$ .

30 14. A process according to claim 7, whereby step (ii) is included and the product is not subjected to a hardening step.

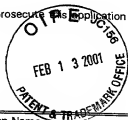
**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (continued)**  
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NUMBER  
F7414(C)

**POWER OF ATTORNEY:** As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute the application and transact all business in the Patent and Trademark Office connected therewith.

CUSTOMER NUMBER: 000201

Direct all correspondence to: CUSTOMER NUMBER 000201



201

Full Name of Inventor	Family Name <b>CHENEY</b>	First Given Name <b>Paul</b>	Second Given Name <b>Edward</b>
Residence & Citizenship	City <u>Wlilp</u> <b>NLX</b>	State or Foreign Country Netherlands	Country of Citizenship United Kingdom
Post Office Address	Post Office Address C/o Iglomora Groep BV, Oia Productie BV, Molenaallee 51	City 7384 AP Wlilp	State & Zip Code/Country Netherlands

202

Full Name of Inventor	Family Name <b>RUSSELL</b>	First Given Name <b>Andrew</b>	Second Given Name -
Residence & Citizenship	City <u>Auckland</u> <b>NLX</b>	State or Foreign Country New Zealand	Country of Citizenship United Kingdom
Post Office Address	Post Office Address C/o Dept of Chemical & Materials Eng, Univ. Auckland, Private Bag 92019	City Auckland	State & Zip Code/Country New Zealand

203

Full Name of Inventor	Family Name	First Given Name	Second Given Name
Residence & Citizenship	City	State or Foreign Country	Country of Citizenship
Post Office Address	Post Office Address	City	State & Zip Code/Country

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201 	Signature of Inventor 202 	Signature of Inventor 203
Date 27/01/01	Date 26/9/2000	Date

**COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY**

(Includes Reference to PCT International Applications)

ATTORNEY'S DOCKET NUMBER  
F7414 (C)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**FROZEN FOOD PRODUCT**

the specification of which (check only one item below):

☐ is attached hereto.☐ was filed as United States application Serial No. \_\_\_\_\_ on \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable)☒ was filed as PCT International application **PCT/EP98/08552** on **23<sup>rd</sup> December 1998**  
and was amended under PCT Article 19 on \_\_\_\_\_ (if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

**PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:**

COUNTRY (if PCT indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
UNITED KINGDOM	9801410.3	22 <sup>nd</sup> Jan 1998	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that /those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code §112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations §1.56 (a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

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U.S. APPLICATION(S)		STATUS (CHECK ONE)		
U.S. APPLICATION NUMBER	U.S. Filing Date	PATENTED	PENDING	ABANDONED
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT APPLICATION NUMBER	PCT Filing Date	U.S. Serial Numbers Assigned (if any)		
PCT/EP98/06552	23 <sup>rd</sup> December 1998			